

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Creatine and Pregnancy Outcomes- A Prospective Cohort Study in Low Risk Pregnant Women: Study Protocol
AUTHORS	deGuingand, Deborah; Ellery, Stacey; Davies-Tuck, Miranda; Dickinson, Hayley

VERSION 1 – REVIEW

REVIEWER	dr. Fares Karamat Amsterdam UMC location Academic Medical Center
REVIEW RETURNED	27-Oct-2018

GENERAL COMMENTS	<p>The research group of Hayley Dickinson has a strong research background regarding creatine and pregnancy. A recent retrospective collaborative study in a pregnant human cohort showed maternal creatine levels appear to be related to fetal growth (Dickinson H, BJOG 2016). This cohort study will provide more information regarding creatine and pregnancy outcomes and might have implication for conducting a clinical trial.</p> <p>I would suggest the authors to add blood pressure measurement as we know that creatine and creatine kinase are associated with blood pressure during pregnancy (Horjus et al. J of Hypertension 2018, 36:000–000, Creatine kinase is associated with blood pressure during pregnancy). I would also suggest the authors to discuss the limitations of this cohort study in the section discussion (which is lacking at this moment).</p>
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REVIEWER	Ozren Polasek Medical School, University of Split
REVIEW RETURNED	09-Nov-2018

GENERAL COMMENTS	<p>Why 8 hours of post-collection waiting? This needs to be reduced. The general feel is that the outcome scope is rather narrow. It is a pity to perform a study of such size and focus on just a single/few traits, I would suggest measuring and collecting more, easily accessible and collectible, relevant data. Why not aiming to over-sample any specific groups at higher risk? Metabolic or otherwise, various disorders might provide more input into homeostasis. Will ethnic diversity affect and dilute the results? Primary outcome measure is "Concentration of maternal blood" sounds very confusing? Clinical variables coverage is rather vague and sounds over-simplified. I would suggest strengthening and focusing some of these definitions to get better inclusion criteria understanding. Urine protocol is confusing - stored at 10 aliquots and then centrifuged? It is better to spin a single tube, aliquot and then freeze. Otherwise, you will have to pool sediment into a separate tube, and that sounds impractical. The timeline of repeated sample collection is poorly explained.</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer #1: Comments to the Author

1. *I would suggest the authors add blood pressure measurement....*

Thank you for this suggestion. Blood pressure readings that coincide with our maternal sampling regime are available, and will be incorporated into our data analysis. Please see below references to blood pressure measurements, as well as other pregnancy events, now made within the study protocol.

Line 46: 'Secondary outcome measures will assess dietary protein intake over pregnancy and any association with maternal creatine, pregnancy events and birth outcomes'.

Line 129: '4. Determine whether there is any association between creatine concentrations across pregnancy and at birth with maternal characteristics in pregnancy and neonatal outcomes, specifically, fetal birth weight and length'.

Line 185-190: 'Socio-demographic data, pregnancy events and birth outcomes data are also collected. Socio-demographic parameters include maternal age, country of birth, ethnicity, and education level. Relevant medical history will capture any pre-existing clinical variables such as hypothyroidism or other correctable nutritional deficiencies. Pregnancy parameters include body mass index (BMI) at booking, blood pressure readings, and gestational weight gain over pregnancy.'

2. *I would suggest the authors to discuss the limitations of this cohort study in the section discussion (which is lacking at this moment).*

Thank you for this suggestion. The following statement has been added to the discussion.

Line 308-313: 'It is beyond the scope of this study to capture all pregnancy populations. As this is a study of low risk pregnant women, it is unlikely to be powered to identify associations between maternal creatine levels and poor pregnancy outcomes. Results will be primarily descriptive; however, data collected in this population may be used to compare to higher risk pregnancy populations in the future'.

Reviewer #2: Comments to the Author

1. *Why 8 hours of post-collection waiting? This needs to be reduced?*

Maternal blood and urine samples are being collected over the course of the day in antenatal clinics, before being transported to the laboratory for processing. Prior to commencing this study, we undertook in house laboratory validation and quality assurance testing to determine whether processing time would affect creatine measurements. We found that creatine concentrations in blood samples, kept on ice, remained stable for up to 8 hours. This time period thus became our upper limit for processing. We have added this detail to the *Sample collection and processing* section of the manuscript.

Line 200: '.....(note: creatine is stable in whole blood, kept on ice, for up to 8 hours)'.

2. *The general feel is that the outcome scope is rather narrow.*

This is the first study to undertake a detailed assessment of the creatine homeostasis during pregnancy. Whilst we are focused on four main objectives, the study design encompasses

a wide-range of analytical techniques that will allow a thorough description of creatine synthesis and metabolism, both in the pregnant women and the placenta. It is also the first study to capture dietary information in relation to creatine homeostasis and identify if any maternal characteristics modify creatine homeostasis. The scope of the project is further enhanced by the generation of the biobank, which will facilitate future research endeavors.

3. I would suggest measuring and collecting more, easily accessible and collectible, relevant data.

Thank you for this comment. We have perhaps not been clear enough in our writings of the scope of the clinical data being captured from this cohort. Please refer to the changes made in the document, which give further detail about the data being collected throughout the study.

Line 185-196: 'Socio-demographic data, pregnancy events and birth outcomes data are also collected. Socio-demographic parameters include maternal age, country of birth, ethnicity, and education level. Relevant medical history will capture any pre-existing clinical variables such as hypothyroidism or other correctable nutritional deficiencies. Pregnancy parameters include body mass index (BMI) at booking, blood pressure readings, and gestational weight gain over pregnancy. Significant antenatal events, include diagnosis of Gestational Diabetes Mellitus (GDM), hospitalisations', enhanced maternal monitoring due to blood pressure changes, or enhanced fetal monitoring due to suspected fetal growth restriction. Labour and delivery outcomes will be captured and will include, type of onset of labour, labour stage time points, drug use during labour and colour of liquor, mode of delivery and blood loss. Neonatal parameters include gestation at birth, gender, apgar scores, weight, height and head circumference and length of hospital stay.'

4. Why not aiming to over-sample any specific groups at higher risk?

Metabolic or otherwise, various disorders might provide more input into homeostasis.

Whilst we could hypothesize that certain metabolic disorders of pregnancy may disrupt creatine homeostasis, there is no evidence at this stage to suggest targeting certain high-risk populations. Thus, our primary objective is to examine low risk 'normal' first, before moving to assess whether creatine homeostasis varies between low and risk pregnancy. Given women are recruited at their first antenatal appointment (10 – 20 weeks), prior to diagnosis of metabolic disorders such as GDM, we anticipate some GDM women will be included in the cohort (as long as they do not meet our subsequent exclusion criteria), and that we will be able to make initial observations (descriptive) around GDM, vitamin D deficiency, iron deficiency etc, within our low risk population.

5. Will ethnic diversity affect and dilute the results?

Australia has a population with great ethnic diversity, thus we feel ethnic diversity within our study population is important for the generalisability of our findings. To date there is no substantive evidence associating ethnicity with changes in creatine metabolism. As such, this will be the first study to assess whether diet and ethnicity may modulate creatine homeostasis over pregnancy. We have included a small amendment to reflect the above comments.

Line 304-307 'This study will enhance our understanding of the potential impact maternal factors, including diet and ethnicity, may have on maternal creatine homeostasis, and whether maternal *de novo* synthesis maintains creatine homeostasis across pregnancy despite variations in dietary intake or maternal characteristics'.

6. Primary outcome measure is "Concentration of maternal blood" sounds very confusing?

Thank you for this comment. We have reworded this section.

Line 167-169: 'Concentrations of creatine, creatine kinase, arginine, glycine and methionine are measured in maternal plasma and urine at 5 time points during gestation, in cord vein and arterial plasma, and placental tissue at birth'.

7. Clinical variables coverage is rather vague and sounds over-simplified. I would suggest strengthening and focusing some of these definitions to get better inclusion criteria understanding.

Thank you for this comment. I hope we have now satisfactorily addressed this issue. Please refer to response to Reviewer 2, Question 3.

8. Urine protocol is confusing - stored at 10 aliquots and then centrifuged? It is better to spin a single tube, aliquot and then freeze. Otherwise, you will have to pool sediment into a separate tube, and that sounds impractical.

Thank you again. This section has been reworded to more clearly explain that we collect urine and centrifuge as one sample, before making aliquots for storage.

Line 267-269 now reads: 'Urine is collected and kept on ice until processing (within 8 hours). The sample is transferred to a 50 mL falcon tube and centrifuged (400g, 20 mins, 4°C), before being aliquoted (10 x 500µl) and stored at -80°C'.

9. The timeline of repeated sample collection is poorly explained.

We have reworded and more clearly articulated the time points of collection across pregnancy.

Line 156-158 '.....After providing informed consent, blood and urine samples and 24-hour food recalls are collected at 5 antenatal visits between 10-20 weeks (time of consent), 21-23 weeks, 24-27 weeks, 28-32 and 33-36 weeks, and at birth (Figure 1).'

Figure 1 has been redesigned to suit the Formatting requirement of BMJ Open.

We have included a paragraph under the Methods Section addressing **Patient and Public Involvement**;

Line 138-141: 'Participants were not asked or offered the opportunity to participate in the study design. The researchers did consider the study requirements in relation to pregnancy care and scheduled all appointments to coincide women's visits to antenatal clinics'.

VERSION 2 – REVIEW

REVIEWER	Ozren Polasek Medical School, University of Split, Croatia
REVIEW RETURNED	22-Nov-2018
GENERAL COMMENTS	Nicely done.